Soil Ecology Project

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Impact of Soil Compaction on Fungi

I have completed this assignment honorably.

Ashton Benfield, Emilia Current, and Gabi Dorsey

Background

Fungi are organisms that live in the soil, decomposing dead material and helping retrieve and recycle nutrients for plants and the other organisms living there (Tugel, 2001). They are quite numerous, with about 907 to 1814 kilograms of biomass in just one acre of land, and while a few of them are harmful, most types are beneficial and even critical to the health of an ecosystem (Dukier, 2004). Two such groups of helpful soil fungi are the saprophytes and mycorrhizae. Saprophytic fungi decompose dead material such as animals and plants and help recycle nutrients including phosphorus, nitrogen, and various micronutrients for plants; while mycorrhizal fungi live symbiotically with the roots of plants (Traquair, 1995 and Tugel/Ingham, 2001).

Saprophytic fungi are also known as decomposers because they transform dead organic material into many things (such as carbon dioxide, organic acids, and fungal biomass) for the other organisms in the soil to consume, and they are also responsible for breaking down the hard to decompose organic materials such as cellulose and lignin (Tugel/Ingham, 2001 and Hoorman, 2016). Saprophytic fungi most commonly live in isolation from other soil organisms, and they can usually be observed covering the remains of dead trees.

Mycorrhizal fungi meanwhile are mutualists that colonize plant roots, retrieve nutrients, provide pathogen protection, and solubilize phosphorus for their host plants to consume and in return the fungi receive carbon for their own metabolic needs (Tugel/Ingham, 2001 and Sikes, 2010). These fungi attach themselves to plant roots and extend microscopic filaments called hyphae that can reach farther than the plant’s longest roots, and because these filaments are smaller than the plant’s roots, they can grow into more compact soil spaces and send water and nutrients to the plant that the plant’s roots otherwise could not access. In return, the plant will provide the fungi with energy in the form of sugars made from the sun's energy and the process of photosynthesis (Nardi, J. B., 2003). Mycorrhizae also solubilize phosphorus for plants to absorb, and this critical element, along with nitrogen, is important to plants. (Busman, 2002). Nitrogen and phosphorus are essential parts of nucleic acids and proteins. Nucleic acids and proteins control chemical reactions within cells. If bacteria are not providing nitrogen and phosphorus to cells, then cells can not produce nucleic acids and proteins, which means that they can not control chemical reactions. If cells do not have the necessary chemical reactions, then they can not survive. The primary and secondary consumers can not get the necessary nitrogen and phosphorus if the plants are not receiving them. If this happens, then the ecosystem will collapse.

The mycorrhizal fungi which help the plants health fall into two main types, endomycorrhizae and ectomycorrhizae. The main difference between the two groups are that endomycorrhizae live within the actual cells of the roots of plants and ectomycorrhizae live outside of the roots in a web-like structure (Mycorrhizal Applications, n.d.). Plant health is essential to the ecosystem, because plants are a vital piece of the food chain. Plants that are associated with endomycorrhizae are a majority of vegetables, shrubs, flowers, grasses, ornamentals, and fruit trees. The plants mainly associated with ectomycorrhizae are mainly conifers, oak trees, and a few woody plants.

Fungi can thrive in forests, but not in anaerobic soil because fungi are aerobic. Soil, though, can become anaerobic through soil compaction (Ouyang, 2016), which occurs when the soil's particles become tightly packed together. This results in a limited amount of oxygen in the soil, which can make it harder for air and water to flow in and out of it (Cotching, 2017). Soil compaction and loosening can be caused by a variety of reasons, which can vary from human to natural influences. Natural soil compaction factors are geologic events such as landslides, earthquakes, avalanches, and certain animals can influence soil compaction and loosening with natural behaviors such as digging, building nests, and running over soil. But humans contribute the most to soil compaction and loosening with actions such as agriculture, construction, and mining (Kyle, 2007).

Most impacts of soil compaction are negative and hurt the plants and organisms in the soil, but there have been a few cases where soil compaction helped the germination of seeds and was able to be beneficial when a period of low-moisture occurred. However with a limited amount of fungi, less nutrients are recycled, roots lose some protection against pathogens and are not colonized. Furthermore, when air and water flow are limited, water drainage is restricted which can raise denitrification, lower plant production, and restrict plant roots from receiving water (Cotching, 2017). Lower plant production will affect the primary consumers because they will not have enough food which will eventually affect the rest of the food chain and ecosystem.

Hence, because soil compaction can have such a potentially damaging impact on fungi, will be testing in our project to see how a specific form of compactions sidewalks, might impact fungi in soil. In order to do this, samples of soil will be taken 0 m, 8 m, and 16 m away from a sidewalk to see if the compaction caused by it impacts fungi growing in the soil. Each plot of soil will be distanced two meters across from the other plot at that distance and will be gathered a total of three times; once each on three separate days. The soil gathered will go through a process of serial dilutions and will be dropped onto fungal plates containing nutrient agar to grow the mold and or yeast in it. Fungus in its nonfunctional form grows as yeast and fungus in its functional form grows as mold. The effects of compaction will be prevalent in the number of mold or yeast spores grown on the fungal plates. We think the more compacted a soil is, the more the density of fungi will decrease in it.

Experiment

I. Problem

How does soil compaction alter the density of fungi in the soil?

II. Hypothesis

The more compacted a soil is, the more the density of fungi will decrease in it.

III. Procedure

A. Independent Variable: the distance from a source of compaction of the sampled soil

B. Dependent Variable: density of soil fungi, and the yeast:mold ratio in the soil

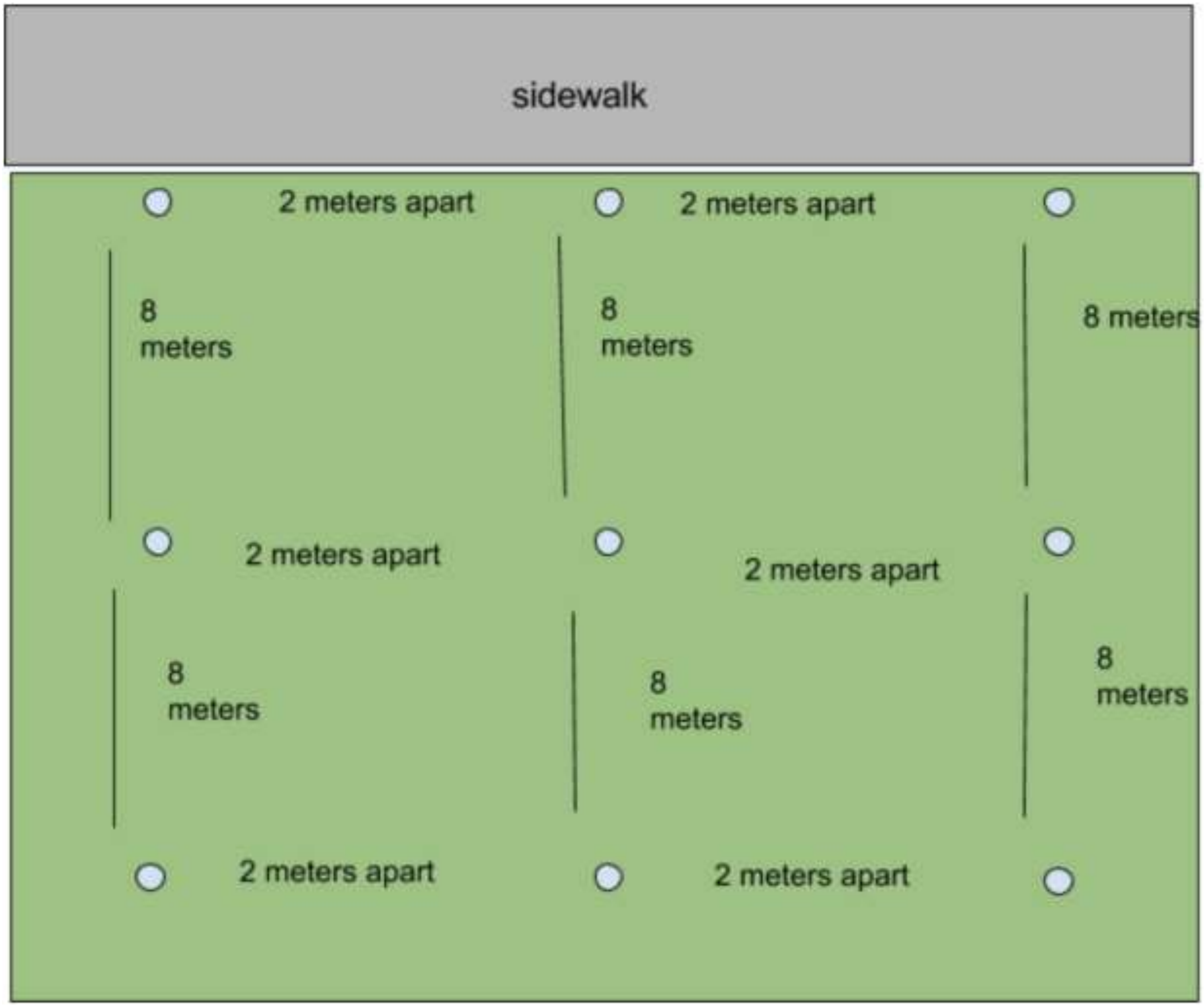
C. Negative Control: samples taken from non-compacted soil

D. Controlled Variable: distance between the columns of soil samples, amount of soil collected, depth of where soil is retrieved, type of soil, plant life on soil, dilutions plated, temperature in which dilutions grow, amount of time dilutions are grown, amount of sterile water, size of transformation tube, soil samples taken on the same day and same time, amount of soil placed into sterile water, degree to which soil is diluted, serial dilutions completed on the same day at the same time, type and amount of nutrient agar, how much soil/water solution on agar plate

E. Step-by-step

1. Mark 9 points with flags within the coordinate of N 39°21.49 and W 76°38.17. The first three are each two meters apart from each other and directly next to the sidewalk. The next three flags should be placed 8

meters away from the flags next to the sidewalk and two meters apart from each other (see diagram). The last three should be 16 meters away from the first three flags next to the sidewalk and two meters apart from each other (see diagram).



*circles are the flagged soil sample locations

2. Take samples all on the same day at the same time: using a soil core extractor, remove 15.5 cm deep of soil with a diameter of 2 cm from each flag-marked point and place each sample into separate correspondingly labeled plastic bags.

3. Perform the following steps 4-14 for serial dilution on the same day at the same time.

4. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label tube "10^0" and (1,1,1) for day 1, row 1, and column 1.

5. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}" and write (1,1,1).

6. Repeat step 5 one more time to one additional 15 ml culture tube labelling them "10^{-2}" and write (1,1,1).

7. Place 1 cc of the (1,1,1) soil sample into the "10^0 (1,1,1)" culture tube.

8. Cap the tube and shake vigorously.

9. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0 (1,1,1)" tube and place into the "10^{-1} (1,1,1)" tube.

10. Cap the tube and shake vigorously.

11. Using the same pipette used in step 9, remove 1 ml of the soil/water mixture from the "10^{-1} (1,1,1)" tube and place into the "10^{-2} (1,1,1)" tube.

12. Cap the tube and shake vigorously.

13. Plate 100 l samples from the 10^0 (1,1,1), 10^{-1}(1,1,1), and 10^{-2} (1,1,1) culture tubes each onto their own separate, correspondingly labeled 3M Petrifilm™ Yeast and Mold Count Plate.

14. Repeat steps 4-13 with the rest of the samples from the same day as the first one that was diluted changing the labels to correspond with the row and column of the soil sample.

15. Allow all plated dilutions to grow for 72 hours.

16. Examine each of the plates for yeast and molds starting with the "10^{-2}" tube first. If at least one yeast is found there, record the total number and dilution. If at least one mold is found there, record the total number and dilution. If no yeast and/or mold is found on "10^{-2}" then

observe "10^{-1}". If no yeast and/or mold is found on "10^{-1}" then observe "10^{0}." After yeasts and molds are found calculate how many fungi were in the original soil with the following equation:

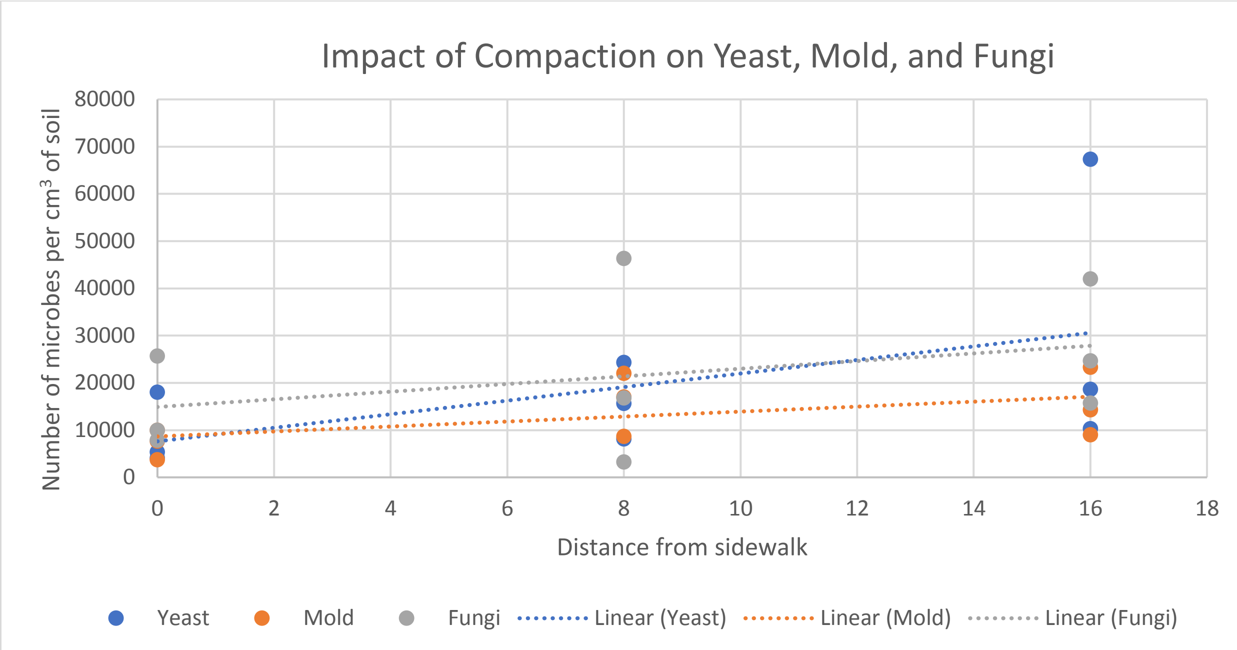
# of Microbes in 1 cc of soil= # of Colonies on sheet x 10^2 x $10^{|\text{dilution at which these colonies were found}|}$

17. Repeat 2-16 two more times on two different days.

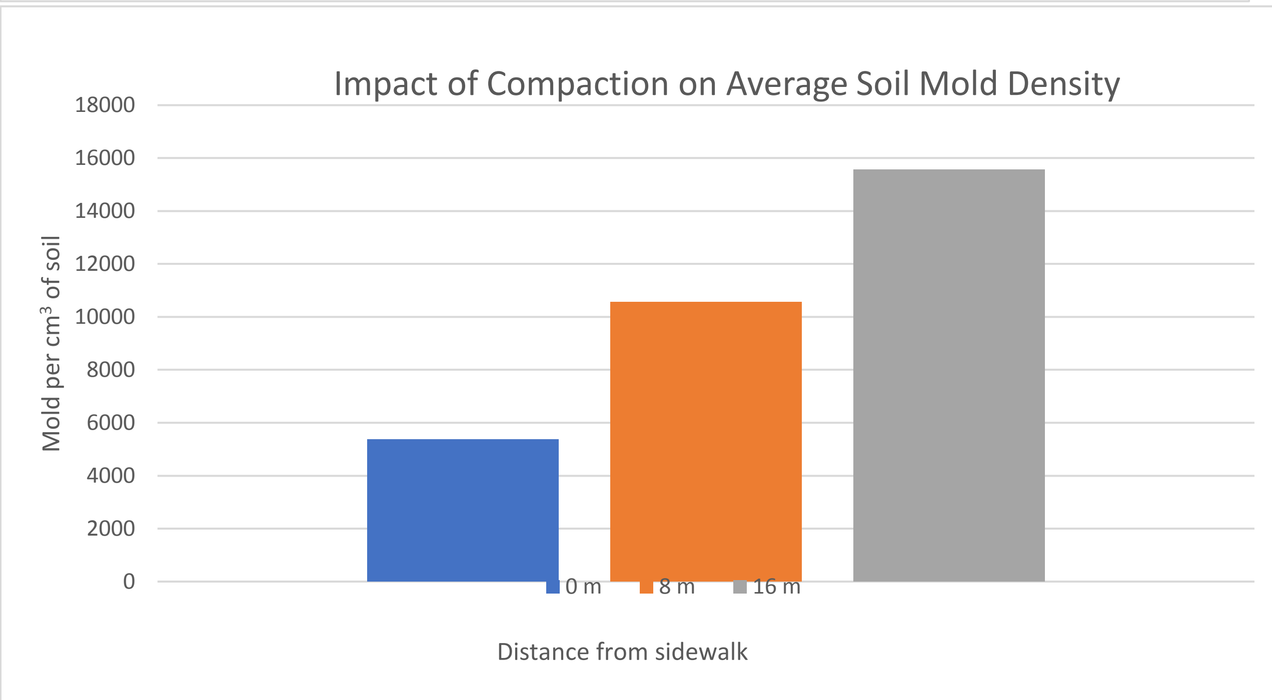
Data

Impact of Compaction on Soil Fungal Density

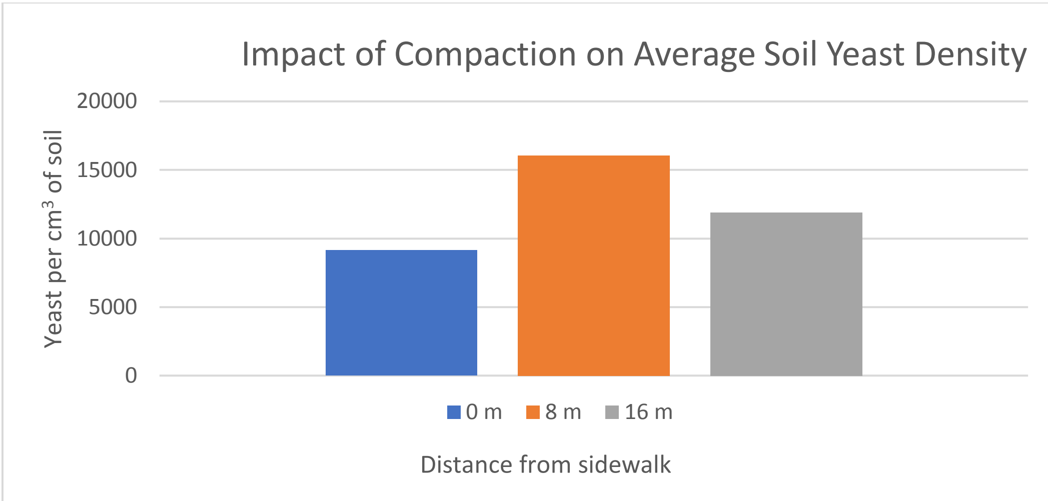
		Distance from Sidewalk								
		0 m			8 m			16 m		
Day	Rows	Yeast Density (#/cc)	Mold Density (#/cc)	Fungi Density (#/cc)	Yeast Density (#/cc)	Mold Density (#/cc)	Fungi Density (#/cc)	Yeast Density (#/cc)	Mold Density (#/cc)	Fungi Density (#/cc)
1	1	20,000	3,000	23,000	3,000	7,000	10,000	1,000	3,000	4,000
	2	14,000	10,000	24,000	4,000	4,000	8,000	10,000	10,000	20,000
	3	20,000	10,000	30,000	40,000	40,000	80,000	20,000	30,000	50,000
Averages		18,000	7,666.67	25,666.67	15,666.67	17,000	3,266.67	10,333.33	14,333.33	24,666.67
2	1	300	1,000	1,300	14,000	10,000	24,000	10,000	5,000	15,000
	2	10,000	10,000	20,000	500	6,000	6,500	10,000	10,000	20,000
	3	2,000	300	2,300	10,000	10,000	20,000	200	12,000	12,200
Averages		4,100	3,766.67	7,866.67	8,166.67	8,666.67	16,833.33	6,733.33	9,000	15,733.33
3	1	3,000	5, 000	8,000	60,000	50,000	110,000	6,000	10,000	16,000
	2	3,000	8,000	11,000	3,000	10,000	13,000	30,000	40,000	70,000
	3	10,000	1,000	11,000	10,000	6,000	16,000	20,000	20,000	40,000
Averages		5,333.33	4,666.67	10,000	24,333.33	22,000	46,333.33	18,666.67	23,333.33	42,000

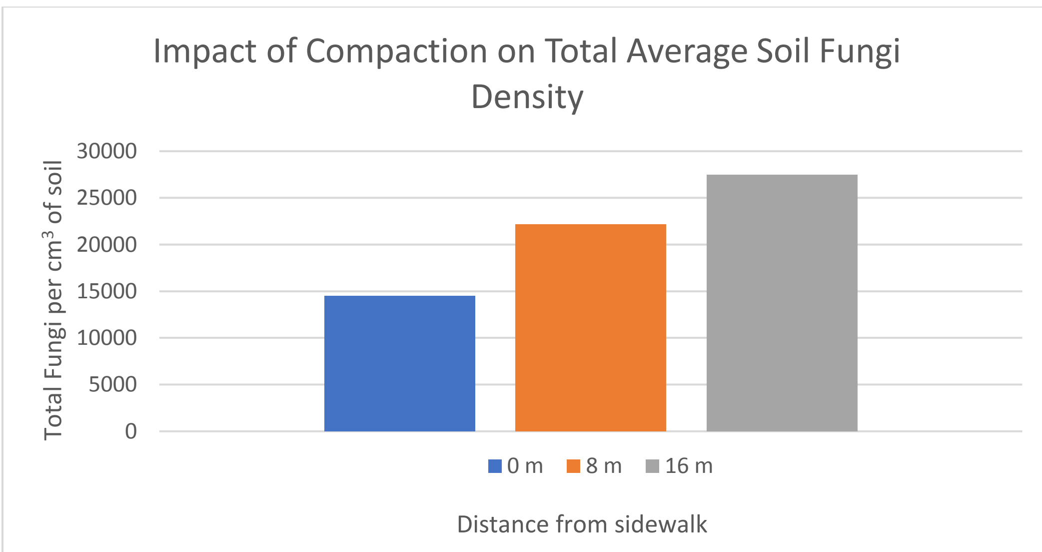


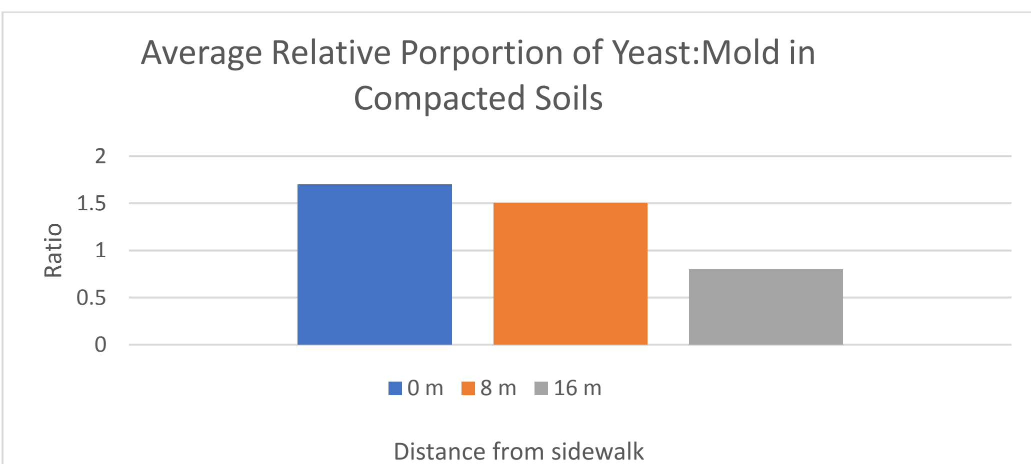
Impact of Compaction on Yeast, Mold, and Fungi
Number of microbes per cm3 of soil
80000
70000
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
12
14
16
18
Distance from sidewalk
Yeast
Mold
Fungi
Linear (Yeast)
Linear (Mold)
Linear (Fungi)



Impact of Compaction on Average Soil Mold Density
Mold per cm3 of soil
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0 m
8 m
16 m
Distance from sidewalk


Impact of Compaction on Average Soil Yeast Density
Yeast per cm³ of soil
20000
15000
10000
5000
0
0 m
8 m
16 m
Distance from sidewalk


Impact of Compaction on Total Average Soil Fungi Density
Total Fungi per cm³ of soil
30000
25000
20000
15000
10000
5000
0
0 m
8 m
16 m
Distance from sidewalk


Average Relative Porportion of Yeast:Mold in Compacted Soils
Ratio
2
1.5
1
0.5
0
0 m
8 m
16 m
Distance from sidewalk

Conclusion

In conclusion, the hypothesis was correct; the more compacted a soil is, the more the density of fungi will decrease in it. The density of average mold cm^3 of soil greatly increased from 0 m to 8 m to 16 m away from the sidewalk. The density of average mold cm^3 of soil at 0 m was 5,366, at 8 m it was 10,555, at 16 m it was 15,555. At 0 m there was less mold because the soil was more compacted than at 16 m which means fungi was not able to live as a mold form. The density of average yeast cm^3 of soil at 0 m was 9,144, at 8 m its density was 16,055, and at 16 m its density was 11,910. The average density of the yeast increases a lot at 8 m. The average fungi per cm^3 of soil was 14,510 at 0 m, 22,144 at 8 m, and 27,466 at 16 m. The linear trendlines for average yeast, mold, and fungi at 0 m, 8 m, and 16 m all increase from 0 m to 8 m and from 8 m to 16 m.

The only data that does not agree with the hypothesis is the density of the average yeast per cm^3 of soil. At 0 m the yeast count was 9,144, then at 8 m it increases to 16,055. The amount of yeast goes back down at 16 m with 11,910. This most likely occurred because of a tree was close to one of the 16 m flags and very close to one of the 8 m flags. The tree did not have any grass growing around it which was mostly a factor of allelopathy. The allelopathy was the reason why the grass could not grow around the tree and it caused an environment in which the fungi could not thrive.

The mold is able to increase from 0 to 8 to 16 m away from the sidewalk because the soil became less compacted with increasing distance from the sidewalk. This is probably because the fungi were are to live in their most functional form which is as a mold.

For future research, tests could be done to see what chemicals the tree that has allelopathy around it is producing. By finding the type of chemicals produced, an experiment can be performed to see how those certain chemicals impact fungi density.

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